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Diagnostic Significance of Urinary Thiodiglycolic Acid as a Possible Tool for Studying the Role of Vitamins B_{12} and Folates in the Metabolism of Thiolic Substances

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Summary

We have found that the determination of thiodiglycolic acid (TDGA) in urine may help to characterize metabolic imbalance of substances participating in methionine synthesis, which leads to hyperhomocystinuria. From the metabolic scheme, based on a proper combination of known facts, we attempted to theoretically explain and to demonstrate the possibilities of TDGA formation *via* different ways of homocysteine transformation. This scheme was used in evaluating the results obtained by testing urine of a woman suffering from impaired function of methionine synthase reductase (CblE type of homocystinuria). The amount of TDGA excreted in her morning urine was very sensitive to the changes in her treatment based upon a combination of N^5-formyl tetrahydrofolate, betaine and vitamin B_{12}. Vitamin B_{12} given in the evening either alone or together with betaine increased the TDGA excretion in the morning urine up to ten times. On the other hand, in the absence of vitamin B_{12}, betaine in combination with N^5-formyl tetrahydrofolate hindered the appearance of TDGA in the morning urine. Generally, the determination of TDGA in urine of an appropriately pretreated patient may indicate the degree of success of the treatment.

Key words

Thiodiglycolic acid (TDGA) • Homocysteine • Urine • Voltammetry • Vitamin B_{12} • Folates • Betaine

Introduction

Thiodiglycolic acid (also called thiodiacetic acid, mercaptodiacetic acid or dicarboxydimethyl sulfide), $S(CH_2COOH)_2$, TDGA-CAS Number 123-93-3), belongs to the large range of physiological products of human metabolism. It is found at concentrations below 20 mg/l in urine of healthy persons. This level increases when the organism is exposed to changes affecting its redox equilibria (Šenholdová-Dlasková 2003, Dlasková *et al.* 2003).

Our research group has developed a simple and accurate voltammetric method of TDGA determination in urine (Dlasková *et al.* 2003). The analysis is preceded by

passing the urine samples through a column filled with microbeads of PVC powder (Dlasková *et al.* 2003). This method and its reproducibility were verified during its evaluation as a possible marker to detect and prevent the exposition of workers to carcinogenic materials in manufacturing polymers polluting air with vapors of vinylchloride monomer (VCM), ethylene oxide, vinylidene chloride, 1, 2-dichloroethane, 1,2-dibromo-ethane, or chloro-alkyl ethers, which have been estimated by other more laborious methods (Šenholdová-Dlasková 2003, Navrátil *et al.* 2004, 2003, Dlasková *et al.* 2000, 2001, 2003, Fenclová 1997, 1998, Heger *et al.* 1982, Samcová *et al.* 1999, Cheng *et al.* 2001). We also used our voltammetric method for determination of TDGA in the urine of persons either taking different drugs as the antihistaminic cetirizine or after consuming various food such as onion (Navrátil *et al.* 2004).

It was proved that TDGA is one of the metabolites of warfare chemical mustard gas (yperite). The appearance of TDGA in microbiological culture during mustard gas degradation was affected by substrate concentration, pH and pO_2 (Ermakova *et al.* 2002).

Similar factors seem to be important for the TDGA formation and its urinary excretion in humans. TDGA levels in morning urine samples of healthy persons were always higher than in the samples collected during the day. Vitamin B_{12}, when administered intramuscularly or in food supplement in the evening, increased the TDGA level next morning. The administration of vitamin B_{12} affects daily TDGA rhythm due to specific metabolic activities (Přistoupilová *et al.* 2005).

S-carboxymethyl-L-cysteine (CMC) is the direct precursor of TDGA. In some experiments in which CMC was administered to volunteers at various time periods, it was found that the metabolic formation of TDGA and its oxidative products was dependent on the time of the day (Hoffman *et al.* 1991, Steventon 1999). In urine samples collected during the night, a part of CMC was transformed into TDGA. In samples collected during the day, sulfoxides (S-O) of CMC and of TDGA were the major components, which originated from the applied CMC. Major part of the CMC was not found, probably because it had been utilized in other metabolic (oxidative) pathways during day time activities (Steventon 1999).

Two-carbon (2C) units, remainders of xenobiotics or oxidative products of amino acids, are further metabolized, bound to cysteine of GSH (glutathione) (Steventon 1999, Laplanche *et al.* 1987). Longer lasting intoxication causes a decrease of cellular GSH resources, which would be otherwise utilized in other natural metabolic pathways (Ambrosi *et al.* 1990). For this reason, CMC as a source of cysteine for GSH synthesis might act as a remedy.

TDGA is not a final product of this pathway, because it is further modified by oxidation (Hoffman *et al.* 1991, Steventon 1999, Ermakova *et al.* 2002). There are principally two possible ways affecting TDGA formation: transmethylation and transsulfuration. Both processes are related to the synthesis and degradation of phospholipids, which are controlled by vitamins folates, B_{12} and pyridoxine. The whole system is dependent on the supply of serine originating from the carbohydrate metabolism.

The present results open the way for further experiments with our voltammetric method to clarify details of TDGA formation and to use its determination as a possible diagnostic and research tool.

Approximately one half of the amount of cysteine necessary for metabolism enters the cells as CMC from food. The other half of cysteine originates from homocysteine (HoCySH) (Mosharov *et al.* 2000), which is released from methionine (methylhomocysteine) by transmethylation. In that reaction, the methyl group is transferred to a suitable acceptor *via* S-adenosyl-methionine (S-AM). HoCySH may then be either remethylated to methionine or used as a source of cysteine *via* cystathionine. Both transformations proceed enzymatically under the complex control of many factors including vitamin B_{12}, folic acid and pyridoxine.

The metabolites of choline, e.g. betaine and dimethylglycine (DMG), are also able to affect these reactions. The enzyme betaine-homocysteine methyl transferase (BHMT) catalyzes the formation of methionine through the transfer of one methyl group from betaine to HoCySH. This type of remethylation depends on folate metabolism as well.

In our present study, we investigated the effect of betaine, vitamin B_{12}, and folate on urinary excretion of TDGA in a woman, here denoted as Case 1, suffering from a rare inborn inability to remethylate HoCySH. Details concerning this patient were described elsewhere (Case 1 – Zavaďáková *et al.* 2002). The aim was to shed more light on this very specific metabolic disorder (Zavaďáková *et al.* 2005).

Methods

Urine samples were collected, stored and

analyzed by the voltammetric technique described in our previous paper (Dlasková *et al.* 2003). The preparation of the sample was done in a column of powdered PVC, urine sample was introduced to the top of the column and eluted by 0.2 M perchloric acid. The resulting eluate was introduced into the electrolytic cell, deaerated by a stream of nitrogen (or other inert gas), and then subjected to voltammetric analysis: accumulation for 10 s under stirring at initial potential of –800 mV vs. SCE, followed by rest period of 15 s and then by potential scan at the rate of –10 mV/s to the final potential of –1200 mV. The temperature was kept constant during measurement within the range 25-35 ^{o}C. The method of double standard addition appeared most appropriate for quantitative evaluation. The analysis was performed by the computer-controlled Eco-Tribo Polarograph using the software Polar 5.1 version for Windows (Eco-Trend Plus, Ltd., Czech Republic) on hanging mercury drop electrode, on mercury meniscus modified silver solid amalgam electrode (Yosypchuk and Novotný 2002) or on solid composite electrode (Navrátil and Kopanica 2002).

The patient "Case 1", now aged 24 years, has been treated basically by betaine ["Cystadane" (Orphan Medical, Canada), containing betaine anhydrous 6-16 g divided into four equal portions for daily application]; folinate ["Calciumfolinat EBEWE 15 mg" (Ebewe Pharma, Austria) containing calcii folinas pentahydricus 19.1 mg in one tablet given daily in the morning]; carnitine ["Carnitene flaconcini orali monodose" (Sigma-Tau, Italy), Levocarnitinum 1 g in 1 tablet – ¼ of the tablet given daily in the morning]; vitamin B_{12} ["Aquo-Cytobion 500 µg inj. sol" (Merck, Darmstadt, Germany) – one injection per week in the evening], and vitamin B-complex Forte (Zentiva, Czech Republic) – Thiamini hydrochloridum 15 mg, Riboflavinum 15 mg, Pyridoxini hydrochloridum 10 mg, Calcii pantothenas 25 mg, Nicotinamidum 50 mg in one tablet given daily in the morning. Detailed changes of the treatment are mentioned in figure legends.

Parallel experiments were done by estimation of TDGA in urine of two healthy young volunteers – a man and a woman. More details are given in the legends to figures. The first volunteer (man, 23 years) was given one dose of vitamin B_{12} in the food supplement "Folic acid, Forte" (Agrochemie, Zlín, Czech Republic) containing 0.2 mg of folic acid and 1 µg of vitamin B_{12} of natural origin in 1 tablet. The second volunteer (woman, 24 years) was given 1 mg vitamin B_{12} i.m. (Léčiva, Czech Republic). Quite incidentally, TDGA was also analyzed in urine of a patient hospitalized for B_{12} deficiency. His urinary TDGA was determined before treatment and after daily repeated i.m. application of vitamin B_{12} (Cyanocobal-aminum 1 mg, Thiamini hydrochloridum 100 mg, Pyridoxini hydrochloridum 100 mg) ["Milgamma N injection" – Wörwag Pharma, Germany)].

During all experiments the patients' meals were checked not to contain excessive amounts of thiolic substances.

The values of TDGA concentration were plotted without correction for creatinine (Přistoupilová *et al.* 2005), which had been previously supposed as necessary to correct the various dilution of urine samples. The correction for specific weight of urine was based on a similar assumption. However, the positions of the maxima and minima of the curves of creatinine and specific weight did not usually correspond to maxima and minima of TDGA (Přistoupilová *et al.* 2005). This was evidently due to different metabolic pathways and excretion rates of creatinine and other substances affecting the specific weight of urine. It was established that the correlation between TDGA values and creatinine concentrations varied in a wide range (correlation coefficient ranged from –0.25 to +0.71). With the probability of 59 % (on average) we can suppose that there exists linear dependence between these two variables (for individual persons the probabilities varied from 10 to 96 %). The highest probability (on average), almost 97 %, was found between creatinine concentrations and specific weight of urine.

When the values, directly affected by the administered B_{12} vitamin, were excluded from these calculations, similar values were obtained (probability of linear dependence of TDGA vs. creatinine reached 64 %, TDGA vs. specific weight 70 %, and creatinine vs. specific weight almost 97 %).

We can conclude that, in general, it is impossible to consider the variables as mutually independent, and that it is impossible to correct the measured values on basis of the presented variables. Therefore, we presented in the figures only the TDGA concentrations alone.

Results

Effect of vitamin B_{12} and of some food supplements on the daily rhythm of urinary TDGA excretion

Healthy volunteers

For the woman (aged 24 years), who received vitamin B_{12} i.m. on the first day in the evening, a small

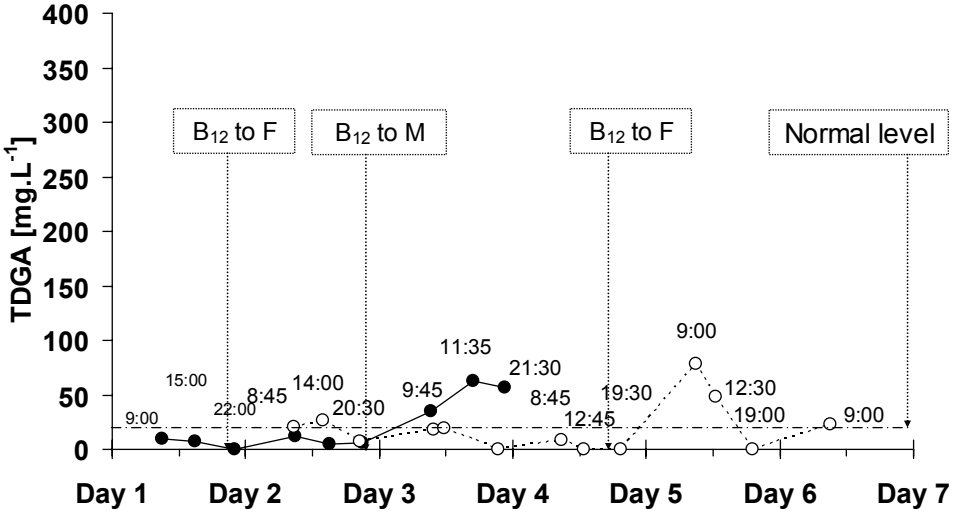

Fig. 1. Effect of vitamin B_{12} on daily rhythm of urinary excretion of TDGA in healthy man (M) (—•—•—) and woman (F) (---o---o----). B_{12} marks the times of B_{12} vitamin administration (M - 1 μg, p.o.; F - 1 mg, i.m.).

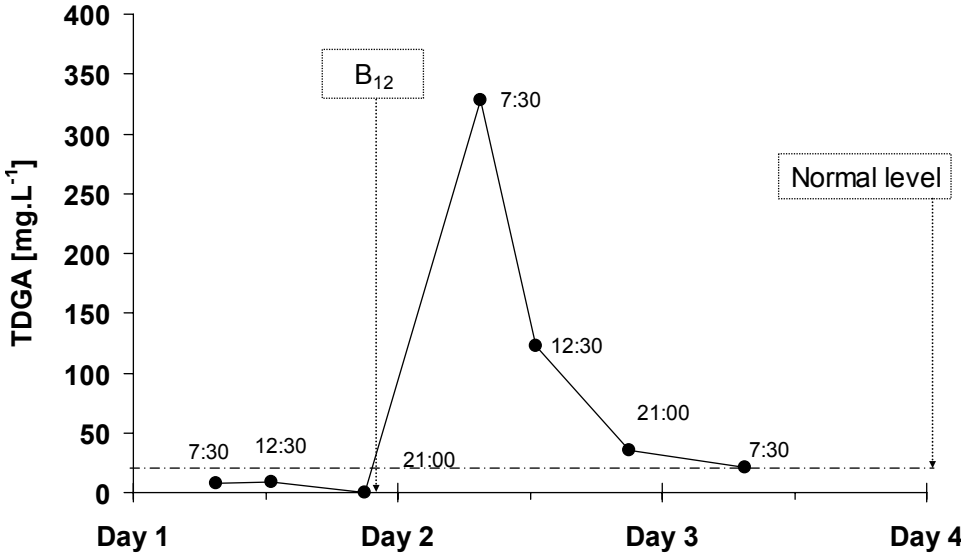

Fig. 2. Effect of vitamin B_{12} on urinary TDGA excretion in the Case 1, treated by betaine and carnitine. B_{12} marks the time of B_{12} vitamin administration (0.5 mg, i.m.).

TDGA increase was found in the afternoon urine next day. The maximum TDGA concentration (78.6 mg/l) was observed in the morning of the 5th day of the experiment after the second application of vitamin B_{12} (Fig. 1). The man (aged 23 years), who took the dietary supplement containing vitamin B_{12} and folic acid in the evening, showed an increased TDGA concentration in the morning urine next day, with a culmination at noon of that day (62.6 mg/l) (Fig. 1).

Man suffering from B_{12} deficiency

The TDGA concentration in the morning urine sample before the beginning of treatment with vitamin B_{12} was 260 mg/l, i.e. 23 times higher than normal value. During the day the TDGA level decreased to zero. Vitamin B_{12} was applied i.m. every evening in course of 5 days. During that time TDGA did not appear in concentrations higher than normal value, up to the morning urine of the 6th day (60 mg/l). The patient felt well and was not followed further.

Case 1 treated without calcium folinate

Basically treated with carnitine, vitamin B-complex and with 16 g betaine (Cystadene) divided in four portions for the daily application, Case 1 did not receive calcium folinate for 2 days. On the 2nd day evening, vitamin B_{12} (Aquo-Cytobion) was applied i.m. and in the next morning 328 mg/l of TDGA was found in

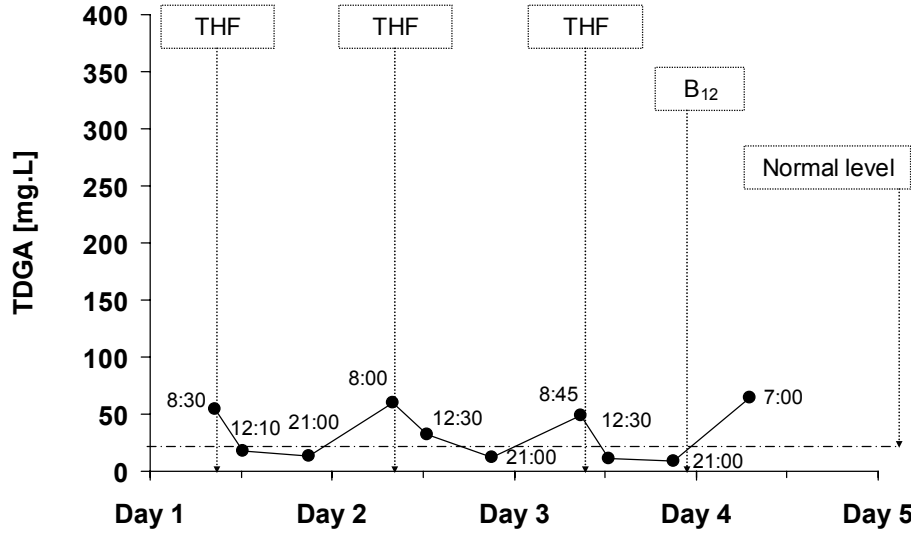

Fig. 3. Effect of vitamin B_{12} on urinary TDGA excretion in Case 1, treated only by calcium folinate (to supply tetrahydrofolate). B_{12} marks the time of B_{12} vitamin administration (0.5 mg, i.m.), THF marks the times of THF administration (15 mg, p.o.).

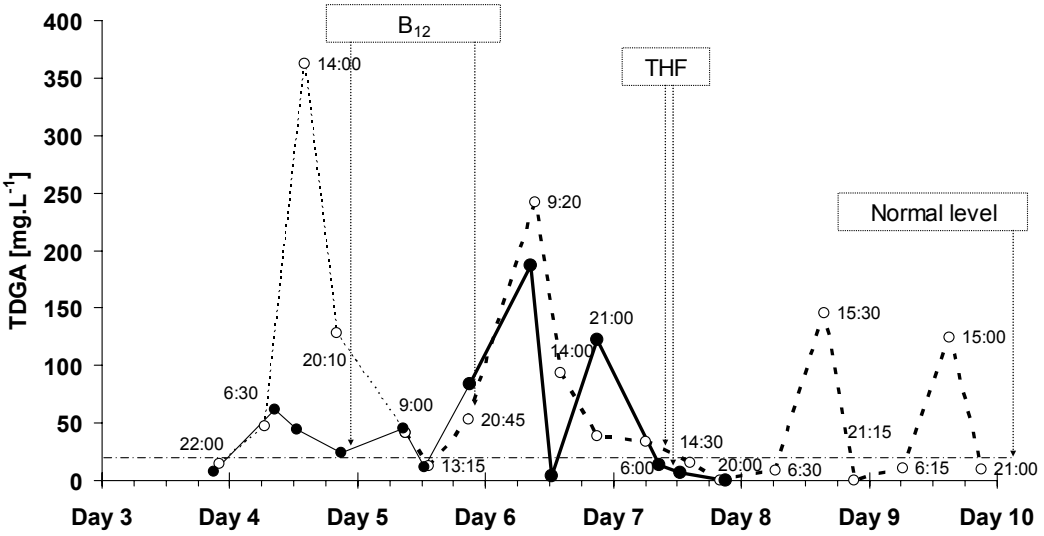

Fig. 4. Changes in urinary TDGA excretion due to different combinations of betaine, vitamin B_{12} and calcium folinate used for treatment of Case 1 in two independent experiments. B_{12} marks the times of B_{12} vitamin administration (0.5 mg, i.m.), THF marks the times of THF administration (15 mg, p.o.). **Set No. 1** Dashed line with empty circles (---o---o----); thin dashed line - without betaine administration; thick dashed - with betaine administration. **Set No. 2** (One month later) Full line with black points (—•—•—); thin line - without betaine administration; thick line - with betaine administration.

the respective urine sample (Fig. 2). During that day, its concentration decreased and it reached normal value on the following morning.

Case 1 treated with calcium folinate without betaine

Basically treated with carnitine and vitamin B-complex, the TDGA levels in morning urine samples, which were determined during three subsequently following days, increased periodically to 50 mg/l or slightly above (Fig 3.). In daytime urine samples the TDGA levels decreased to normal. Intramuscular application of vitamin B_{12} on the 3rd day evening caused only a slight increase to 65 mg/l TDGA in the morning urine on the next day.

Case 1 treated with different combinations of betaine, vitamin B_{12}, and calcium folinate

Basically treated with carnitine and vitamin B-complex, Case 1 obtained neither calcium folinate nor vitamin B_{12}, nor betaine since the 1st day in two independent sets of experiments (Fig. 4). During the 4th and 5th days of both sets, the morning TDGA values were above normal level. In set No. 1 the afternoon value of TDGA on the 4th day reached 362 mg/l. The treatment

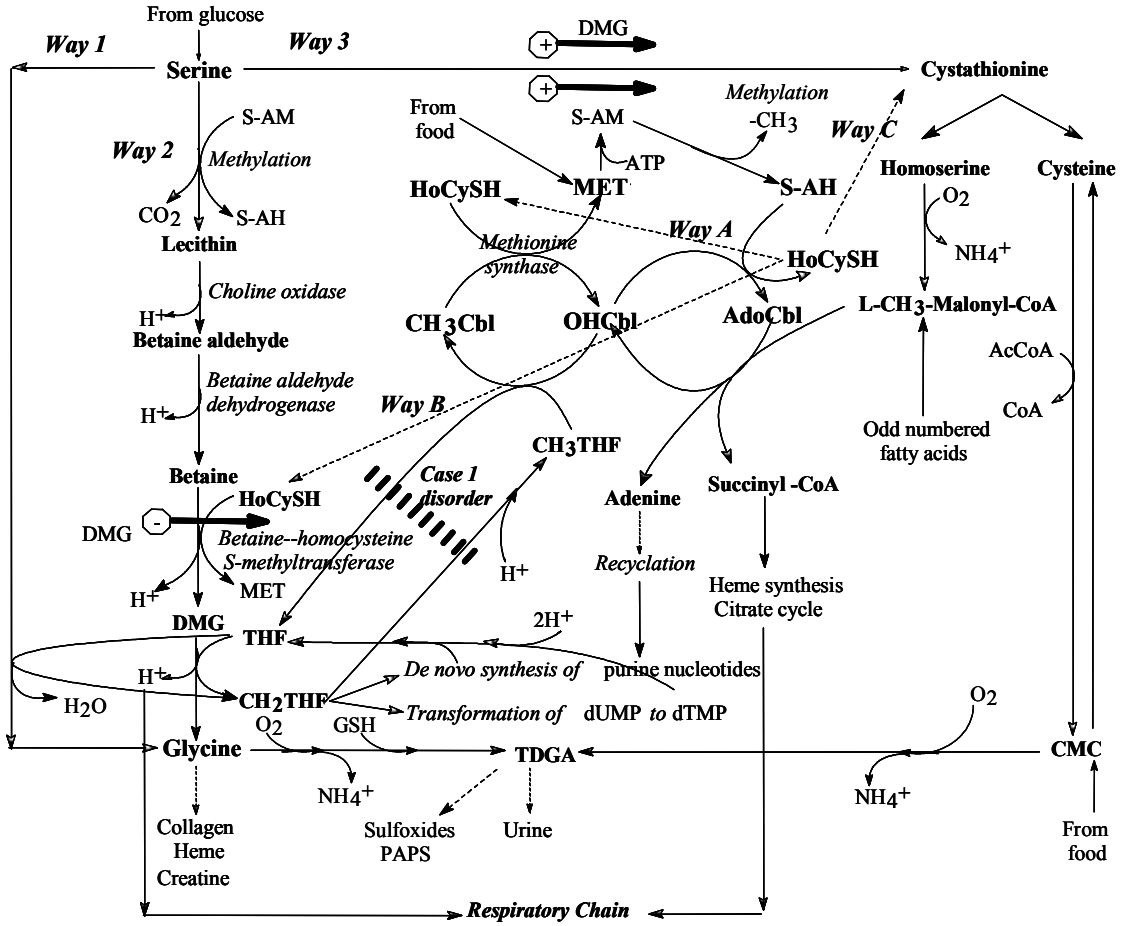

Fig. 5. Scheme of metabolic pathways including TDGA, homocysteine, folates and cobalamin participation

with betaine started in this set in the morning of the 5th day, before i.m. vitamin B_{12} administration in the evening of the same day. The TDGA level increased in the urine in the next day morning to 242 mg/l. During treatment with betaine alone, TDGA values in the afternoon urine on the 8th and 9th day increased to 130 and 110 mg/l, respectively.

In set No. 2, vitamin B_{12} was given i.m. in the evening of the 4th day, 24 h before betaine administration on the 5th day. TDGA increased in the next day morning urine to 170 mg/l, then it dropped to zero in the afternoon and increased again in the evening urine.

In both sets of experiments (No. 1 and No. 2), the administration of calcium folinate together with betaine in the morning of the 7th day hindered TDGA excretion into urine during the whole day.

Discussion

Considerations about the changes of TDGA in urine of the studied persons helped us to better understand the role of vitamin B_{12} in metabolic processes.

Case 1 was, according to genetic studies, denoted as CblE type of homocystinuria. Until now, only 17 patients with this disorder have been described worldwide. Identification of mutations in fibroblasts of nine European CblE patients, one of which is Case 1, confirmed the hypothesis that defects in methionine synthase reductase are the cause of the CblE type of homocystinuria. Eight different mutations were identified (Zavaďáková *et al.* 2005). Case 1 was classified as folate-dependent, but not cobalamin-dependent (blood HoCySH levels from 50 to 90 μmol/l) (Zavaďáková *et al.* 2002)).

Case 1 is treated with N^5-formyltetrahydrofolate (leucovorin) which is usable only after the transformation to coenzymatically active THF forms (Slavík 1962). Case 1 is evidently able to form such variants necessary for synthesis of purine and pyrimidine bases, because she has no problems with proteosynthesis (Fig. 5, Way 1).

Case 1 is further treated in parallel with betaine which is a normal metabolite in the pathway of decomposing phospholipids or acetylcholine (Fig. 5, Way 2). For the formation of both latter mentioned substances from serine, three molecules of S-AM are

necessary. AdoCbl and HoCySH might be produced (in the reaction with OHCbl) from these three molecules of S-adenosylhomocysteine (S-AH) (Orendáč *et al.* 2003, Niklasson 1983) for Way A, Way B, Way C (Fig. 5). Betaine, applied as remedy, has already three methyl groups bound to nitrogen. It is not yet clear to what extent the application of betaine does influence the synthesis of phospholipids and acetylcholine *de novo* (the anabolic part of Way 2).

The aim of the treatment of Case 1 is to remove HoCySH surplus by means of the enzyme BHMT and simultaneously to synthesize sufficient amount of methionine and S-adenosylmethionine for methylations. During this process, BHMT transfers one of the three methyl groups of betaine to HoCySH. Thus methionine and dimethylglycine (DMG) are formed in the catabolic part of Way 2 (Fig. 5). The remaining two methyl groups of betaine, now as part of DMG, react with THF to form methylenetetrahydrofolate (CH_2THF). The simultaneously released H^+ and e^- enter into the respiratory chain to produce energy (Murray *et al.* 2003). Enzymes dimethylglycine dehydrogenase (EC 1.5.99.2) and sarcosine dehydrogenase (EC 1.5.99.1) transfer 4 H^+ and 4 e^- directly into the respiratory chain (Murray *et al.* 2003)**.** Glycine, the end product of Way 1 and 2, is widely used in metabolism (heme, collagen, creatine, glutathione etc.).

It has been found that kidneys are important for the formation of methionine from HoCySH via BHMT (http://www.expasy.org/cgi-bin/nicezyme.pl?2.1.1.5) and of GSH from CMC (Zhao *et al.* 1995). BHMT activity is monitored by mutual molecular ratios of HoCySH, betaine, DMG and folates in blood. Low concentration of folates and abundance of HoCySH and DMG inhibit methionine synthesis from HoCySH by BHMT feed-back inhibition (McGregor *et al.* 2001) (Way 2, Fig. 5). In addition, the increased level of DMG activates the reaction between HoCySH and serine through cystathionine synthase (CS) (Way 3 and Way C, Fig. 5). This leads to the transformation of serine and HoCySH to homoserine, and to cysteine and to its derivatives (Way 3 and Way C). However, the increased level of DMG slows down simultaneously the production of CH_2THF in Way 2 and probably also in Way 1. The products of oxidation of CH_2THF (nethenyl THF and N^{10}-formyl THF) are necessary for the *de novo* synthesis of nucleotides. Decreased production of CH_2THF in Way 2 due to increased concentration of DMG leads to a decreased flow of protons and electrons to the respiratory chain and consequently to a decreased production of energy.

It is known that vitamin B_{12} supports oxidative transformations of serine and glycine in liver (Murray *et al.* 1993). These reactions lead to glycolic acid, which conjugates with cysteine (e.g. from GSH or CMC) to form TDGA (Dlasková *et al.* 2003, Ambrosi *et al.* 1990).

In our scheme (Fig. 5) we show three different degradative pathways of serine (denoted as Way 1, Way 2 and Way 3) and of HoCySH (denoted as Way A, Way B, and Way C). All are under the control of vitamin B_{12} and folates. Way 1 and Way 2 lead to glycine, Way 3 to cysteine. The latter way is identical with Way C of HoCySH. The common oxidative end-product of cysteine is inorganic sulfate. It is excreted from the body or used after reaction with ATP as active sulfate (PAPS) for collagen and sulfolipid syntheses or detoxication. TDGA is an intermediate near the end of the oxidative pathways of glycine and cysteine, controlled by vitamins mentioned above. When the metabolism of thiolic substances and of 2C units is equilibrated, TDGA is not excreted into urine as was proved in our experiments.

The increased level of TDGA in urine brought about by metabolic disorder in Case 1 sheds more light upon concerted interrelationships among vitamin B_{12}, folates and DMG, which all affect the course of the metabolic ways illustrated in Figure 5 and discussed above. Case 1 is not able to form CH_3THF from CH_2THF. For this reason the transformation of OHCbl to CH_3Cbl is out of order. Moreover, there is lack of THF and therefore the first part of Way 2, connected with building up of lecithin (choline), is partially depressed, as well as other reactions dependent on remethylation, especially the metabolism of neurotransmitters. Hence, there is shortage of THF needed for transformation of DMG to glycine in Way 2. In absence of THF, DMG (formed from betaine) accumulates and activates CS (McGregor *et al.* 2001). In Case 1, the high level of urinary TDGA after administration of B_{12} and betaine in the absence of THF might be a result of increased release of cysteine from HoCySH and simultaneously of increased oxidation of glycine due to the activation of CS (Figs 2 and 4).

Betaine, the source of DMG, given to Case 1 in absence of folates, caused the excretion of TDGA at any time of the day, also due to unclear composition of food (Fig. 4). Therefore, more cysteine and more oxidative products could be formed. We may consider in general that increased TDGA levels indicate disturbance of the normal degradation pathway of cysteine and of 2C units.

It seems also that in humans the appearance of TDGA in urine is a sign of imbalance between substrate offer and oxygen consumption, similarly as it had been observed in bacteria (Ermakova *et al.* 2002). Both, surplus or lack of vitamin B_{12} caused an increase of TDGA excretion in the morning urine. It corresponds to the experiments with CMC (Steventon 1999). It is evident that the mentioned daily rhythm consists of two basic phases. One is connected with increased TDGA excretion during resting time in the night, whereas the other is connected with disturbance in thiolic metabolism, caused by meal or drug consumption and by daily activities. The lower supply of O_2 to tissue cells in the night and faults in cysteine transformation into its active derivatives is accompanied by the decrease of the flow of protons and electrons into respiratory chain. Similar effects of barbiturates and diazepines on respiratory chain also increase the TDGA excretion (Sharma *et al.* 1980). In Case 1, THF alone (Fig. 3), or even better in combination with betaine (Fig. 4), prevented the increase of urinary TDGA levels caused by addition of vitamin B_{12}. This was probably due to the stimulation of Way 1 and Way 2 leading to the formation of 2C units, which originate from glycine.

Case 1, when treated either with folic acid, vitamin B_{12} or betaine separately, exhibited increased level of TDGA. If Case 1 was given the mentioned drugs in feasible combination, TDGA was not detectable in urine (Fig. 4). It is evident that the determination of TDGA can help to find the optimal combination of drugs for curing diseases related to disorders in enzyme activities, which are involved in pathways described in Figure 5.

Our study indicates that a lot of problems are open for further detailed investigations. It can be speculated that the metabolic disorders of Case 1 do not cause trombosis, the typical health problem usually accompanying hyperhomocysteinemia (Zavaďáková *et al.* 2005), because her Way 1 and Way 3 operate evidently well and her body is sufficiently saturated with sulfur containing substances.

Way 1 (probably most common in nature) enables the transformation of serine to glycine and supplies CH_2THF for the synthesis of purine nucleotides *de novo* and for the transformation of dUMP to dTMP. It seems that in Case 1 there is probably an imbalance in this process in bone marrow cells. This metabolic disorder is ascribed to macrocytic anemia, which accompanies hyperhomocysteinemia in Case 1.

Activities of all enzymes shown in our scheme (Fig. 5) do affect the reduction of CH_2THF to CH_3THF and the further fate of CH_3 group in the region of methione synthase activity. From this point of view, it can be explained why each of 9 patients (Zavaďáková *et al.* 2005) with impaired methionine synthase reductase declared as CblE type of homocystinuria, had different health problems.

Conclusions

In Case 1 there is an inborn defect of the cooperation between vitamin B_{12} and folates (Zavaďáková *et al.* 2002). Both vitamins existing in their different coenzymic forms enable the progression of many vital enzymatic reactions. A characteristic metabolic disorder in Case 1 is the inability to transform the folic acid (F) into active tetrahydrofolate (THF). Moreover, she is unable to form the methyl group bound to THF. These defects endanger principally her life, because methyl tetrahydrofolate (CH_3THF) is the unique form of folate in blood of healthy humans. Consequently, Case 1 cannot transfer the methyl group to vitamin B_{12} and thus cannot form methylcobalamin (CH_3Cbl), the main cobalamin derivative in human blood (Matthews 1979). The insufficient supply of methylated forms of both vitamins and their altered turnover in brain cells might be the cause of her neurological disorders.

Changes of TDGA concentration, determined by the simple voltammetric method in urine, indicate imbalance in cooperation of enzyme activities, taking part in the release and transport of protons and electrons into the respiratory chain. The resulting decrease of energy production is in general one of the causes of this metabolic syndrome.

The determination of TDGA in urine and the proposed simplified metabolic scheme (including methionine synthase) (Fig. 5), might be helpful in the search for the loci of the fault in mutual interactions between thiolic substances, vitamins B_{12}, betaine and folates.

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Abbreviations

2C	Two-carbon unit
AdoCbl	Adenosyl-cobalamin
ATP	Adenosine triphosphate
BHMT	Betaine-homocysteine S-methyltransferase (EC 2.1.1.5)
CH_2THF	N5,10-Methylenetetrahydrofolate
CH_3Cbl	Methylcobalamin
CH_3THF	Methyltetrahydrofolate
CMC	Carboxymethylcysteine
CS	Cystathionine synthase (EC 2.1.1.5 or EC 2.1.1.48)
DMG	Dimethylglycine
dTMP	Deoxythymidine monophosphate
dUMP	Deoxyuracil monophosphate
F	Folic acid
MET	Methionine
MS	Methionine synthase (EC 2.1.1.13)
OHCbl	Hydroxycobalamin
PAPS	3'-Phosphoadenosine-5'-phosphosulfate (active sulfate)
pO_2	Partial pressure of oxygen
S-AM	S-adenosylmethionine
S-AH	S-adenosylhomocysteine
S-O	Sulfoxide
TDGA	Thiodiglycolic acid
THF	Tetrahydrofolate

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